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RNA Folding Studies

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ABSTRACT: Synchrotron X-ray-dependent hydroxyl radical footprinting was used to probe the folding kinetics of the P4-P6 domain of the Tetrahymena group I ribozyme, which forms a stable, closely packed tertiary structure. The 160 nt domain folds independently at a similar rate ($\sim 2 \text{ s}^{-1}$) as it does in the ribozyme, when folding is measured in 10 mM sodium cacodylate and 10 mM MgCl_2 . Surprisingly, tertiary interactions around a three-helix junction (P5abc) within the P4-P6 domain fold at least 25 times more rapidly ($k \gg 50 \text{ s}^{-1}$) in isolation, than when part of the wild type P4-P6 RNA. This difference implies that long range interactions in the P4-P6 domain can interfere with folding of P5abc. P4-P6 was observed to fold much faster at higher ionic strength than in 10 mM Naocacodylate. Analytical centrifugation was used to measure the sedimentation and diffusion coefficients of the unfolded RNA. The hydrodynamic radius of the RNA decreased from 58 Å to 46 Å over the range of 0-100 mM NaCl. We propose that at low ionic strength, the addition of Mg^{2+} causes the domain to collapse to a compact intermediate where P5abc is trapped in a non-native structure. At high ionic strength, the RNA rapidly collapses to the native structure. Faster folding most likely results from a different average initial conformation of the RNA in higher salt conditions.